INNOVATIVE VECTOR TOOLS

Bergen, Norway

Dr. Beate Stern, CSO

info@unitargeting.com

INNOVATIVE VECTOR TOOLS

UTR
UNITARGETINGRESEARCH

Improving mammalian cell-based protein expression –
over the entire bioproduction continuum:

- Drug development (time-saving)
  - ✓ improved developability
  - ✓ up-scalable
- Any protein/cell line, with focus on
  - ✓ mAbs, monomers, difficult-to-express proteins
  - ✓ CHO cells
- Drug manufacturing (cost-saving)
  - ✓ increased protein yield
  - ✓ correct protein processing

INNOVATIVE VECTOR TOOLS

UTR
UNITARGETINGRESEARCH

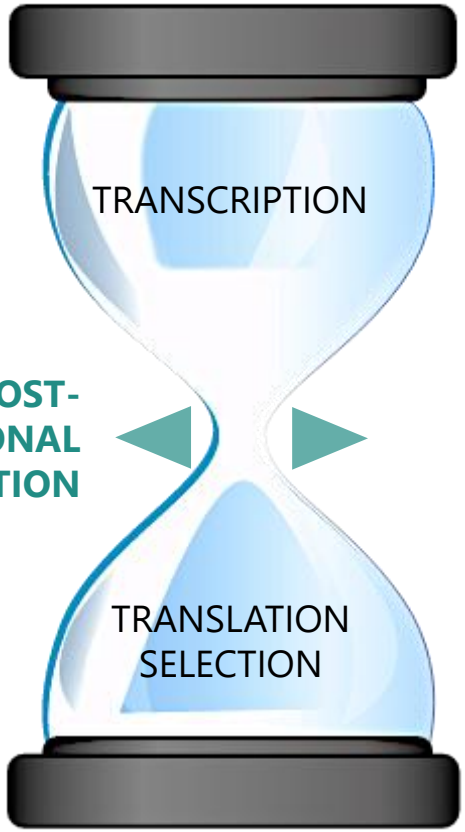
Improving mammalian cell-based protein expression –
through a unique scientific approach:

- Focus on
  - ✓ efficient post-transcriptional regulation
  - ✓ effective protein entry into the secretory pathway
  - ✓ bioinformatics-aided design
- Benefits
  - ✓ compatible with any vector system
  - ✓ additive effect on other expression technologies
  - ✓ customisation for individual proteins

INNOVATIVE VECTOR TOOLS

UTR UNITARGETINGRESEARCH

UTR® vector tools address the **bottleneck** of protein expression.



POST-TRANSCRIPTIONAL REGULATION

Additive effect √

Expression vectors focus normally only on:

TRANSCRIPTION

- strong promoter to boost transcription
- chromatin opening elements to stabilise and enhance transcription
- targeted integration into hot spots
- gene amplification
- high stringency selection markers

TRANSLATION, SELECTION

- codon optimisation of the sequence encoding the POI
- efficient ribosomal recruitment
- FACS-assisted pool enrichment of high-yielding cells
- automated clone selection

Overview of the Presentation

UTR
UniTargetingResearch

I. UTR Tool Box
II. Scientific Background
III. Proof-of-Concept
IV. Tools and Case Studies
V. Patents and Licenses

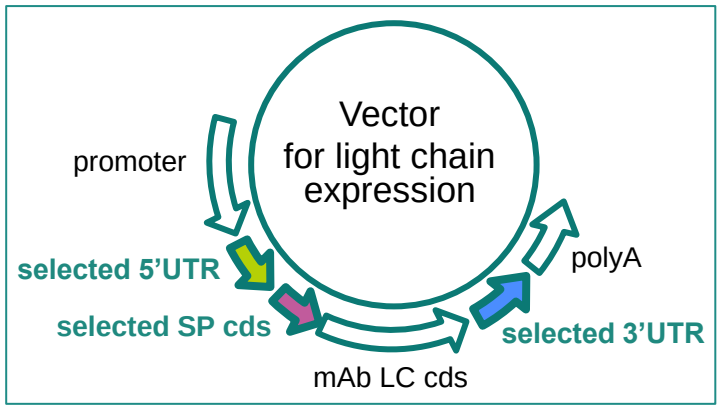
I. UTR Tool Box

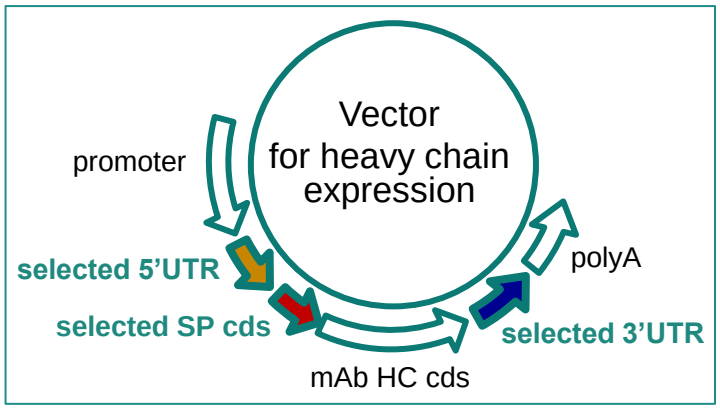
UniTargetingResearch

Efficient protein synthesis/secretion is dependent on

- specific genetic regulatory elements flanking the coding sequence (cds) of the protein of interest (POI):
  - ✓ **signal peptide (SP) cds**
  - ✓ **5′ untranslated region (5′UTR)**
  - ✓ **3′ untranslated region (3′UTR)**

- the appropriate combination of the specific elements in the expression vector (for e.g. mAb expression)





I. UTR Tool Box

UTR
UniTargetingResearch

UTR® Betatech

Optimal regulatory element combination for efficient protein synthesis and secretion in general, to improve the customer's production platform.

- ✓ Optimised non-identical element combinations for mAb LC/HC

UTR® Protech

Computationally designed mutant of a selected SP to improve both yield and quality of a specific POI.

- ✓ Any POI, particularly difficult-to-express proteins

I. UTR Tool Box

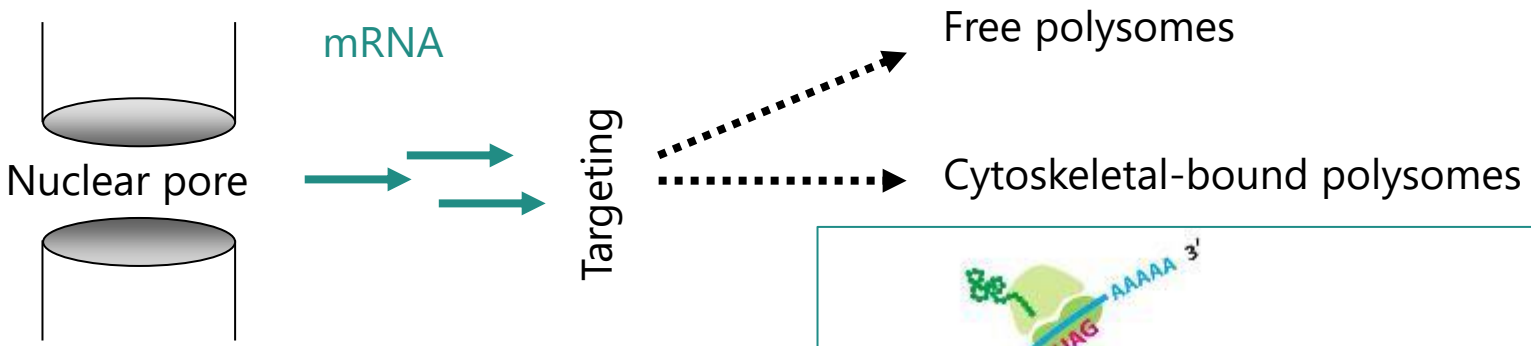
UTR UniTargetingResearch

Manner of implementation and utilisation of the UTR vector tools

	UTR®Betatech	UTR®Protech
Provided *in silico*	√	√
Stand-alone technology	√	√
Optionally in combination	√	√
Production platform improvement	√	
Protein specific		√
Protein class specific	√	
Applicable as in-house tool kit	√	
Exclusivity		√ (customer's SP)

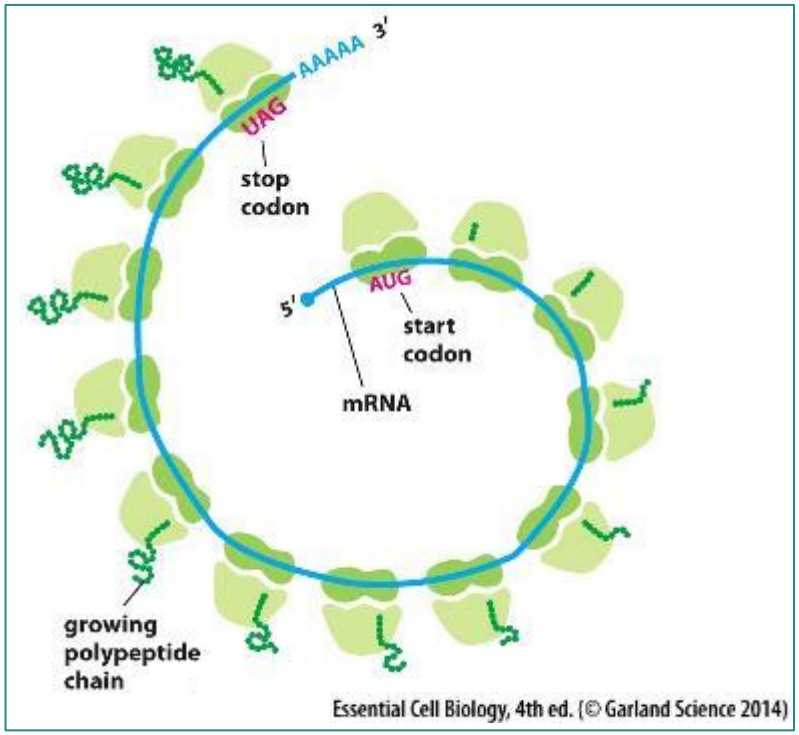
II. Scientific Background

UTR UniTargetingResearch



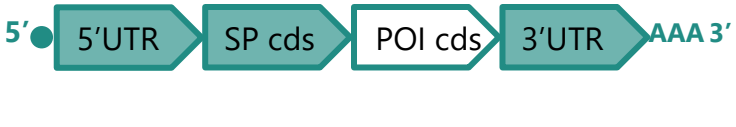
mRNA localisation is a means of targeting protein synthesis to specific cellular compartments.

mRNA is directed to free polysomes and cytoskeletal-bound polysomes for synthesis of intracellular proteins.

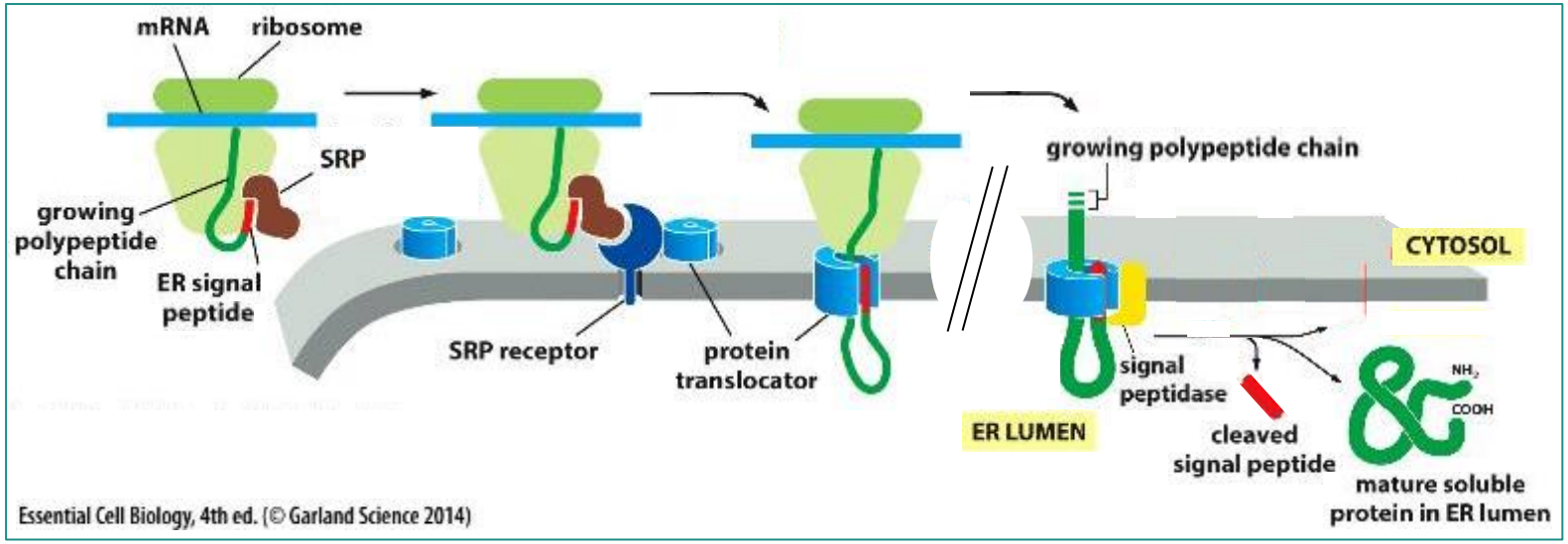


II. Scientific Background

UTR
UniTargetingResearch

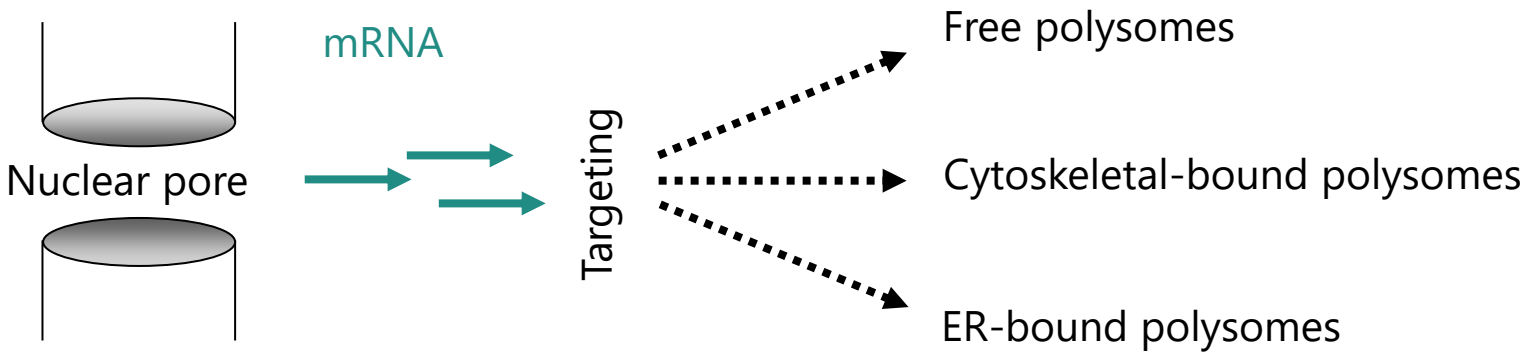


mRNA is directed to endoplasmic reticulum (ER)-bound polysomes for the synthesis of membrane and secreted proteins.



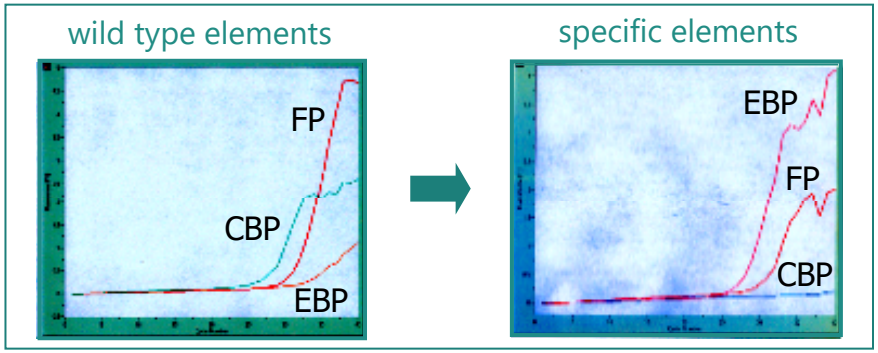
II. Scientific Background

UTR UniTargetingResearch



mRNA encoding an intracellular protein could be re-directed to the ER by using appropriate regulatory elements (5'/3' UTRs, SP cds).

This triggered the idea to exploit the elements for high-level protein synthesis/secretion.



mRNA in polysome fractions of CHO.

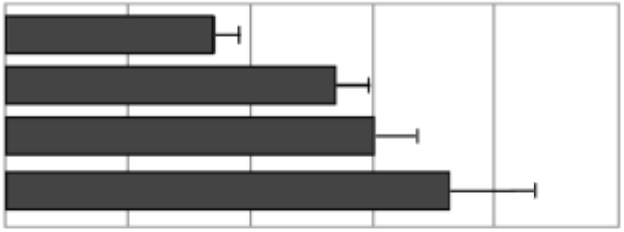
FP, free polysomes; CBP, cytoskeletal-bound polysomes; EBP, ER-bound polysomes

III. Proof-of-Concept

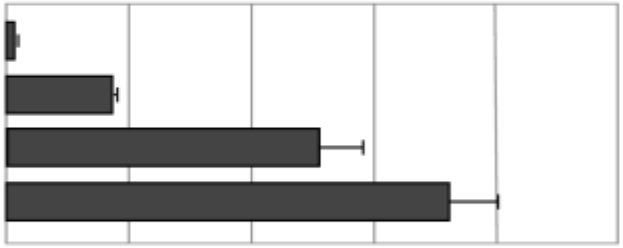
UTR UniTargetingResearch

Model protein (luciferase) in medium from transfected CHO cells

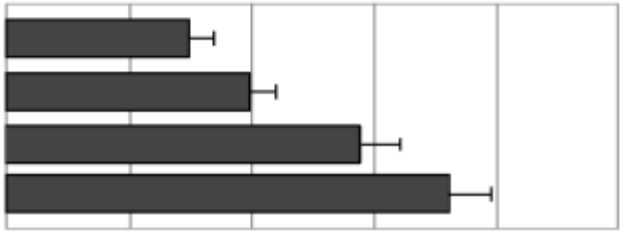
Effect of different 5'UTRs



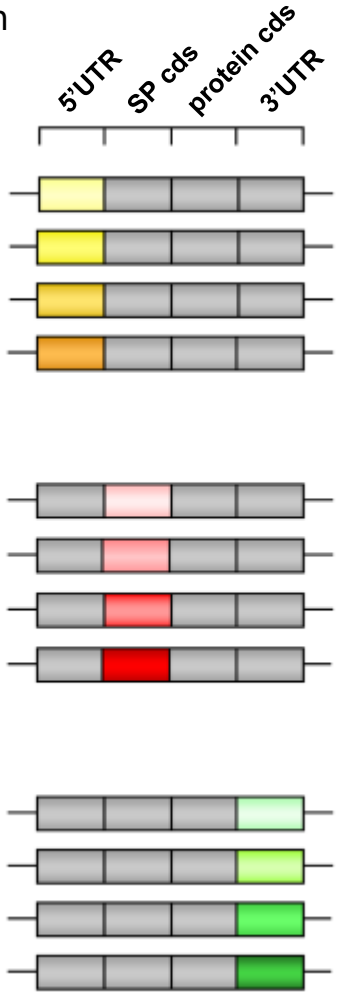
Effect of different SPs



Effect of different 3'UTRs



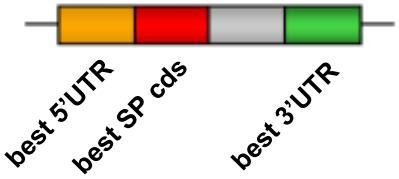
Relative enzyme activity



Vector constructs

Vectors differing only in the individual elements indicated (colours)

Defining UTR®Tech

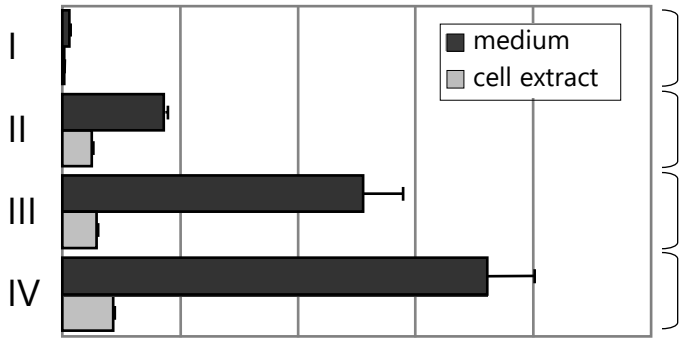


III. PROOF-OF-CONCEPT

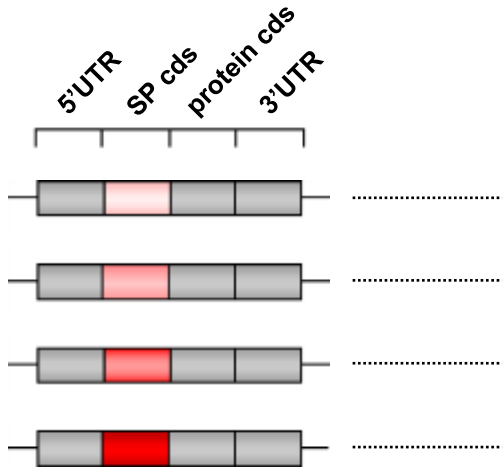
UNITARGETINGRESEARCH

Model protein in medium/cell extract sampl from transfected CHO cells

Effect of different SPs

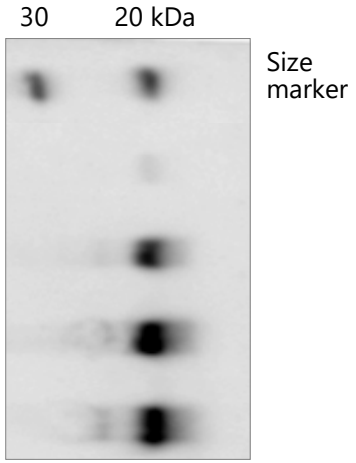


Relative enzyme activity



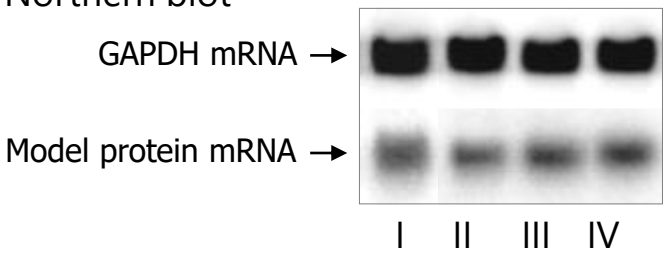
Vector construct

SPs from: I, human albumin; II, human interleukin-2; III, human trypsinogen; IV, *Gaussia princeps* luciferase



Western blot (medium samples) results correlate with activity measurements.

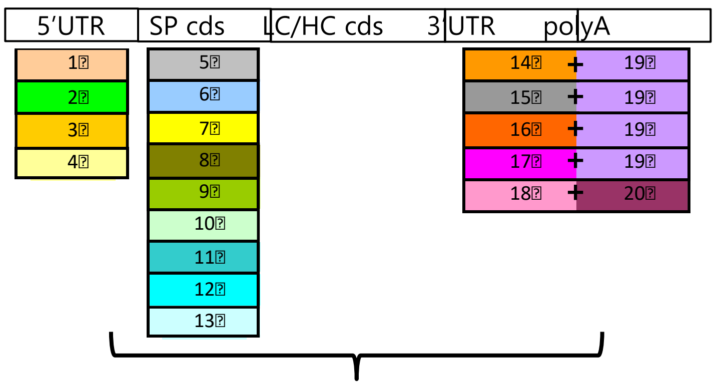
Northern blot



A large variation in product level (factor 50) but similar amounts of mRNA demonstrate that the observed differences in protein synthesis and secretion are the result of **post-transcriptional regulation**.

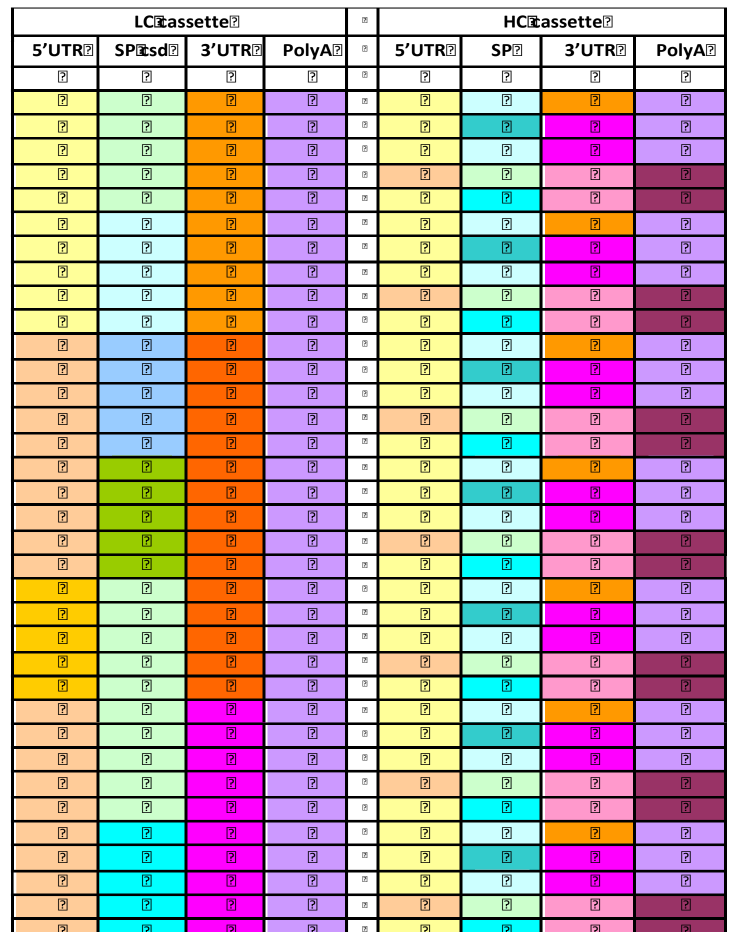
III. Proof-of-Concept

Next step: a combinatorial approach* to identify optimal combinations of selected high-performing elements for mAb production in CHO cells



All possible element combinations were tested for mAb LC and HC expression, respectively.

The best performing LC- and HC-expression cassettes were then combined in all possible ways and tested for mAb production.



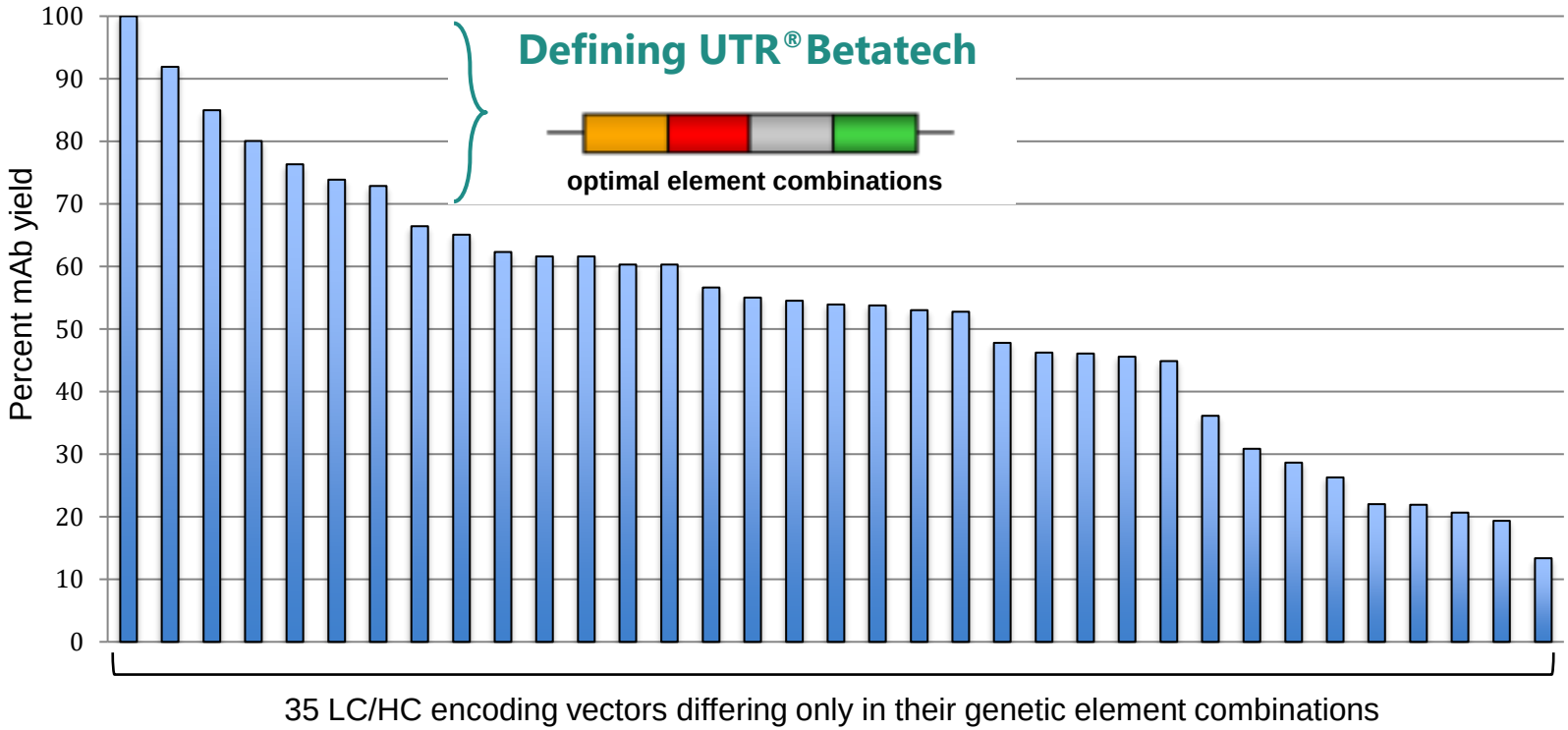
*study in collaboration with Novartis

III. PROOF-OF-CONCEPT

UTR UNITARGETINGRESEARCH

Next step, *cont.*: 7,5 fold range in mAb yield

Productivity of cell pools transfected with the LC/HC-encoding vectors in shake flasks under generic batch culture conditions. The results were verified with a second mAb.



IV. Tools and Case Studies

UTR UniTargetingResearch

UTR® Betatech

Principle

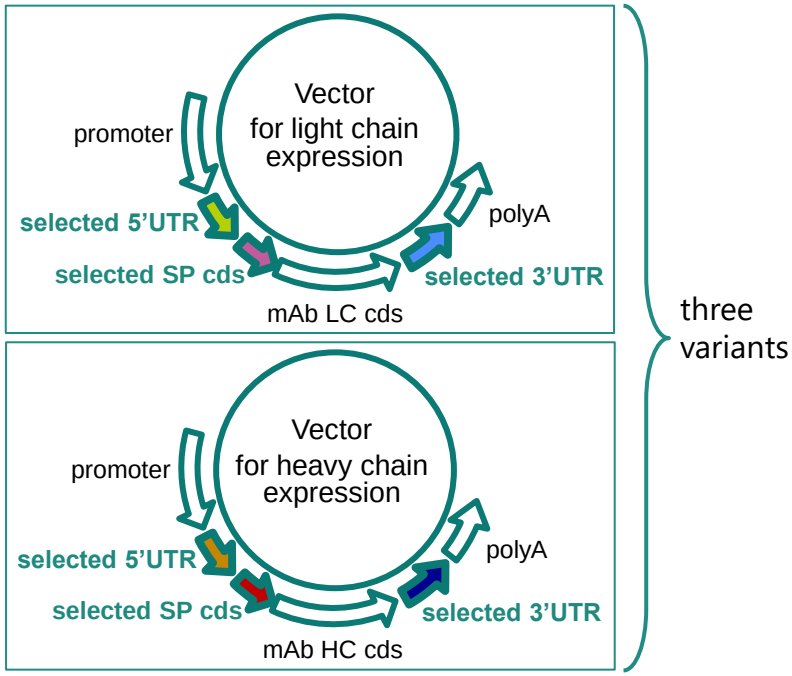
Optimal regulatory element (5'UTR, SP, 3'UTRs) combination for efficient protein synthesis and secretion.

Tool

Comprises a panel of three selected variants of element combinations.

Application

- ✓ Any POI
- ✓ To be incorporated into the customer's vector
- ✓ Identification of the best variant in the customer's manufacturing platform



Single-gene vectors for mAb LC/HC, with optimised non-identical element combinations.

Advantages

- ✓ Overall improvement of customer's production platform to achieve higher yields
- ✓ Can be used as a "tool kit" by the customer

IV. Tools and Case Studies

UTR UniTargetingResearch

UTR®Betatech – Results from selected industrial studies

Global biopharmaceutical company*

- ✓ Pools in ambr® fed-batch culture reached mAb yields >4 g/L and cell specific productivities >20 pg/cell/day.
- ✓ Titer and Qp considered high under the given conditions and regarding the specific mAbs chosen by Novartis.

Global contract manufacturer*

- ✓ With mAbs up to 5.6 fold increase of mean titer and 3.2 fold increase of mean Qp as compared to their reference (pools in 14-days fed-batch culture).
- ✓ With Fc-fusion proteins up to 2.3 fold increase in mean titer and 2.0 fold increase in mean Qp as compared to their reference (pools in 14-days fed-batch culture).

Further studies

- ✓ Other proteins and cell lines and various selection systems.

*studies performed with CHO cells

IV. Tools and Case Studies

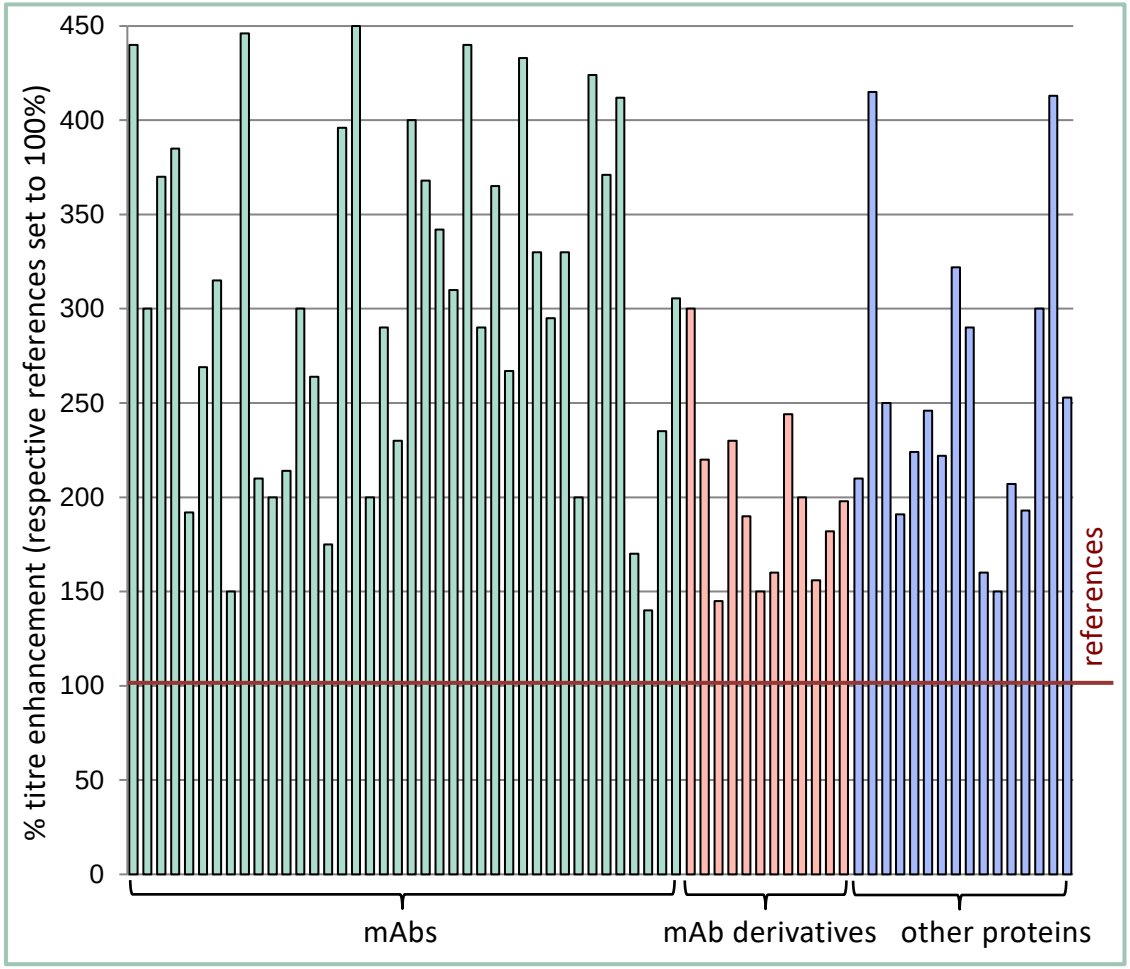
UTR UniTargetingResearch

UTR® Betatech – Result overview from various studies

As shown, compared to the respective references, typical titre enhancements achieved were

- ✓ 200% for mAbs;
- ✓ 100% for mAb derivatives (e.g. FC- fusion proteins);
- ✓ 150% for other commercially relevant proteins (e.g. hormones, enzymes).

Host cell lines used were CHO (mainly), HEK-293 or Hep G2, and selection markers DHFR or GS.



IV. Tools and Case Studies

UTR UniTargetingResearch

UTR®Protech – the SP challenge

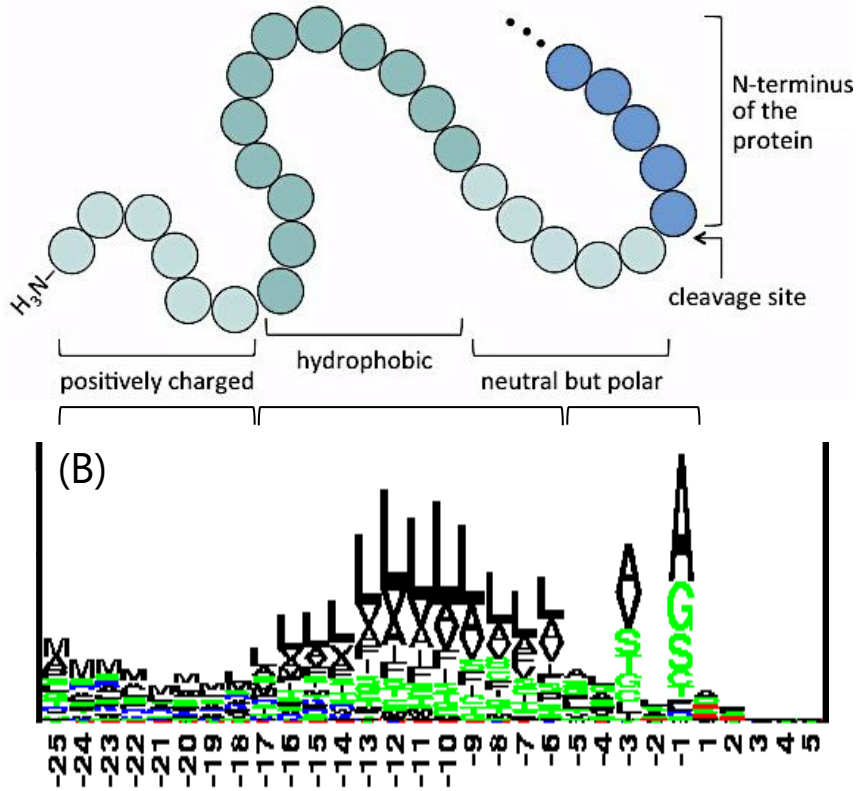
SPs have a common structure, but no consensus sequence, indicating their important regulatory role in

- mRNA/protein targeting
- protein ER-translocation
- protein processing

The SP structure-function relationship is unknown.

UTR has established an algorithm which

- ✓ allows the prediction of SP functionality
- ✓ enables SP upgrade by mutant design
- ✓ permits the improvement of SPs for specific proteins



(A) An SP where circles represent amino acids. (B) The height of each letter showing the relative abundance of the corresponding amino acid in eukaryotic SPs. (from http://www.cbs.dtu.dk/services/SignalP-4.1)

IV. Tools and Case Studies

UTR UniTargetingResearch

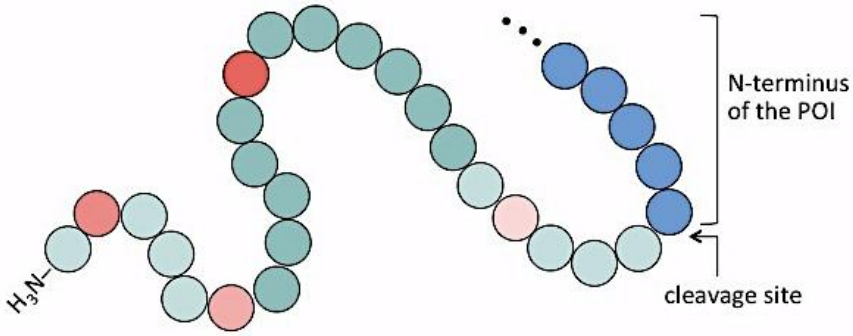
UTR® Protech

Principle

Computationally designed mutant of a selected SP to improve both yield and quality of a specific POI.

Tool

Comprises defined mutants of a given SP, meeting a high "acceptance threshold" regarding performance and SP-cleavage fidelity.



Customised SP mutant exemplifying positions where amino acids are replaced with those shown in red. Considering the N-terminus of the POI tailors the SP mutant to the specific POI.

Application

- ✓ Any POI, particularly difficult-to-express proteins
- ✓ Any chosen SP, including our superior proprietary SP (derived from *Gaussia princeps*)
- ✓ To be incorporated into the customer's vector

Advantages

- ✓ Unique yield/quality-enhancing SP mutants tailored for specific POIs
- ✓ Only the N-terminal sequence of the POI needs to be disclosed

IV. Tools and Case Studies

UTR UniTargetingResearch

UTR®Protech – Results from industrial case studies

In-house

- ✓ Our algorithm predicts high SP performers.

Global biopharmaceutical company

- ✓ Up to 4 fold titer increase with monomeric proteins, after subjecting a given SP to protein-specific “Protech mutagenesis”.

IV. Tools and Case Studies

UniTargetingResearch

Summary

UniTargetingResearch AS provides unique vector optimisation tools to enhance production of biotherapeutics and other proteins.

Main benefits are:

- ✓ the focus on post-transcriptional regulation, which is largely disregarded by other high-level expression approaches;
- ✓ the focus on protein-specific SPs and thus customised vectors for precise SP cleavage and high-level expression;
- ✓ the large achievable yield margin, i.e up to 750% as demonstrated;
- ✓ the complementarity with other high-level expression approaches;
- ✓ the versatility, making it easy to implement the technologies in any production platform;
- ✓ the broad application range, including many market segments of the biopharmaceutical arena.

V. Patents and Licensing

UTR UniTargetingResearch

IP

UTR's intellectual property comprises patents and know-how. The outstanding performances achieved are the result of both the optimal combination of UTR's proprietary genetic elements, and UTR's expertise about optimal vector composition and components with regards to the customer's expression platform.

Patent portfolio

- WO2005001099* (*Gaussia princeps* SP)
  - granted in Europe (patent no. EP 1639111)
  - granted in USA (patent no. US 8067198)
  - granted divisional in USA (patent no. US 9115364)
  - granted in India (patent no. IN 246957)

*exclusive license being granted to UniTargetingResearch AS by patent holder

- WO2010038145 (UTR®Tech/UTR®Betatech)
  - granted in Europe (patent no. EP 2344524)
  - granted in USA (patent no. US 9018001)
- WO2011018766 (UTR®Tailortech/UTR®Protech)
  - granted in Europe (patent no. EP 2464728)

V. Patents and Licenses

UTR
UniTargetingResearch

UTR commercial models

The UTR licensing model is flexible and can be adapted to the customer's revenue projections. It comprises 3 core elements:

1) Project service

Best results are obtained if UTR experts are involved in the design/modification of the customer's expression vector. Suggested designs may include both UTR tools and other non-proprietary genetic elements.

2) Evaluation license

The evaluation of UTR tools are to be performed in the customer's lab according to a study protocol provided by UTR.

3) Commercial license

A positive outcome will result in a commercial license to be structured in accordance with the customer's business model.

- Large pharma a ONE–OFF license fee, if desired;
- Drug development companies a yearly license fee;
- Contract manufacturers per project;